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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicants: Markl et al.

Serial No.: 09/699,243

Filed: 27 October 2000

For: METHYLATION ALTERED DNA SEQUENCES AS MARKERS ASSOCIATED WITH HUMAN CANCER

Examiner: Jeanine A. Goldberg

Art Unit: 1634

Docket No.: 47675-14

Date: 23 May 2003

Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria Virginia 22313-1450

**AFFIDAVIT OF DR. CATHY LOFTON-DAY UNDER 37 C.F.R. § 1.132**
(IN SUPPORT OF RESPONSE UNDER 37 C.F.R. § 1.116 AND REQUEST FOR CONTINUED EXAMINATION UNDER 37 C.F.R. § 1.114)

Sir:

I, Dr. Cathy Lofton-Day, being duly sworn, say:

1. I am substantially familiar with the above-identified patent application, and am aware that it discloses and claims novel diagnostic methods comprising determination of the methylation state of particular CpG dinucleotide sequences that are differentially methylated between or among genomic DNA samples, including, for example between or among genomic DNA samples corresponding to normal and cancerous tissue.

2. I am an internationally recognized scientist and am presently employed as Director of Molecular Biology at Epigenomics, Inc., Seattle, WA (from January 2001 to present). I received a Bachelors Degree in Biology in 1977 from College of Charleston, Charleston, SC,

and a Ph.D. degree from Medical University of South Carolina, Charleston, SC in 1989.

3. I am an author or co-author of more than twenty peer-reviewed research articles and my research has been presented at numerous national and international meetings. My curriculum vitae is attached hereto as APPENDIX A.

4. In my capacity as a Research Director and molecular biologist, I am familiar with a broad spectrum of screening methods and assays, including diagnostic assays, based on analysis of differential methylation of CpG dinucleotide residues in genomic DNA, and with identifying and characterizing statistically significant trends and correlations relating to DNA methylation in normal and diseased tissues, including cancer tissues and transformed cell lines (*see* attached resume, APPENDIX A).

5. I understand that one or more claims of the above-referenced patent application remain rejected under 35 U.S.C. § 112, ¶ 1, on the grounds that one skilled in the art would not know how to use the claimed invention, because the claimed invention is allegedly not described in such a way as to *enable* one skilled in the relevant art to make and/or use the invention as a diagnostic assay. I generally understand that a patentable diagnostic utility in the present sense requires a statistically significant correlation between methylation and disease states.

6. I have overseen experiments that confirm the diagnostic utilities taught and disclosed in the present patent application with respect to SEQ ID NOS:36 and 37 in the additional contexts of breast and colon cancer as summarized in **Table 1** below:

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**TABLE 1. Confirmation of Diagnostic Utility of Subject Marker Sequences**

| MARKER FRAGMENT | EXPERIMENTS (MS-APPCR) | | | STATISTICALLY-SIGNIFICANT DIAGNOSIS (Y/N) |
|---|---|---|---|---|
| | Number of experiments | Sample number | Cancer type | |
| SEQ ID NO:36 | 2 | 35 | breast | Yes (all experiments); diagnostic hypermethylation |
| SEQ ID NO:37 | 2 | 1(pool) | breast | Yes (all experiments); diagnostic hypermethylation |
| | 3 | 4 | colon | |
| | 1 | 10 | prostate | |

7. Specifically, as shown in Table 1, the marker sequences represented by SEQ ID NOS:36 and 37 are hypermethylated in a statistically significant sampling of breast, colon and prostate cancers as indicated. These results confirm, as originally taught and disclosed, that these sequences have a specific, credible and substantial utility as diagnostic markers for cancer.

8. In the experiments of Table 1, the method of Methylation-sensitive Arbitrarily-primed Polymerase Chain Reaction (MS-APPCR; described by Gonzalgo et al., *Cancer Research* 57:594-599) was used to characterized the genome using methylation sensitive restriction enzymes and CG-rich primers. Significantly, this is the same method that was used in the above-identified patent application to show utility for prostate cancer, and further confirms the teachings and conclusions thereof. Results for the prostate samples were confirmed using Methylated CpG Island Amplification (MCA; as described by Toyota et al., *Cancer Res.* 59:2307-12, 1999, and in WO 00/26401A1).

9. Therefore, a person of ordinary skill in the art would reasonably conclude, as originally taught and disclosed in the above-identified application, that there is a specific, credible and substantial cancer diagnostic utility for the instant marker sequences.

10. I further declare that all statements made herein of my own knowledge are true and that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code.

Cathy Lofton-Day

State of Washington)
) ss.:
County of King)

On this 23 day of May 2003, before me, a Notary Public in and for the State aforesaid, personally appeared Cathy Lofton-Day, to me known and known to me to be the person of that name, who signed and sealed the foregoing instrument, and she acknowledged the same to be her free act and deed.

Lui Kay Baker
Notary Public

Commission expires 3-31-04

# Catherine E. Lofton-Day, Ph.D. - CURRICULUM VITAE

Epigenomics, Inc.
1000 Seneca St., Suite 300
Seattle, Washington 98101
clofton@us.epigenomics.com

206-883-2913-office
206-254-9151-fax

## Educational Background

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|---|---|---|---|
| College of Charleston, Chas., S. C. | BS | 1977 | Biology |
| Medical University of S. C. | Ph.D. | 1989 | Molecular & Cellular Biology and Pathobiology |

## Career History

| | |
|---|---|
| 2001-present | EPIGENOMICS, INC., Seattle, WA |
| | **Director of Molecular Biology** |
| 1997-2001 | ZymoGenetics, Inc., Seattle, WA |
| | **Associate Research Director, Bioinformatics** |
| 1996-1997 | ZymoGenetics, Inc., Seattle, WA |
| | **Senior Scientist, Protein Discovery** |
| 1991-1996 | ZymoGeneticS, Inc., Seattle WA |
| | **Scientist, Molecular and Cellular Biology** |
| 1990-1991 | ZymoGenetics, Inc., Seattle WA |
| | **Post-doctoral Fellow, Molecular and Cellular Biology** |
| 1989-1990 | Oregon Health Sciences University |
| | **Post-doctoral Fellow, Dept. of Cell Biology & Anatomy** |
| 1985-1989 | Medical University of S.C. |
| | **Pre-doctoral fellow, Dept. of Pharmacology** |
| 1980-1985 | Medical University of S.C. |
| | **Research Assistant, Dept. of Pediatrics** |
| 1978-1980 | Medical University of S.C. |
| | **Research Technician, Dept. of Pathology** |

**Patents (awarded)**

1. Methods of Detecting Phospholipid Transfer Activity and Kits Therefor. Joseph R. Day, John J. Albers, **Catherine E. Lofton-Day**, Janet L. Adolphson. ZymoGenetics, Inc. USA; University of Washington. Patent # 5,610,019, issued March 11, 1997.

2. Phospholipid Transfer Proteins and DNA Encoding Them. Joseph R. Day, John J. Albers, **Catherine E. Lofton-Day**, Janet L. Adolphoson. ZymoGenetics, Inc. USA; University of Washington. Patent # 5,622,843, issued April 22, 1997.

3. Human semaphorin zsmf7. James L. Holloway and **Catherine E. Lofton-Day.** ZymoGenetics, Inc. USA, Patent #WO9945114A2, issued November 4, 1999.

4. Testis-specific Insulin Homolog Polypeptides. Si Lok, Darrell Conklin, Robyn L. Adams. Anna C. Jelmberg, **Catherine E. Lofton-Day,** Stephen R. Jaspers. ZymoGenetics, Inc. USA. Patent #5,959,075, issued September 28, 1999.

5. Purified Thrombopoietin and Method of Making It. John Forstrom, **Catherine Lofton-Day,** Si Lok. ZymoGenetics, Inc. USA. Patent #5,986,049, issued November 16, 1999.

6. Methods for Stimulating Granulocyte/Macrophage Lineage Using Thrombopoietin. Richard D.Holly, Si Lok, Donald C. Foster, Frederick S. Hagen, Kenneth Kaushansky, Joseph L. Kuijper, **Catherine E.**

**Lofton-Day**, Pieter J. Oort. ZymoGenetics, Inc. USA; University of Washington Patent #5,989,537, issued November 23, 1999.

7. Testis-specific Insulin Homolog Polypeptides. Si Lok, Darrell Conklin, Robyn L. Adams. Anna C. Jelmberg, **Catherine E. Lofton-Day,** Stephen R. Jaspers. ZymoGenetics, Inc. USA. Patent #6183991, issued Feb. 6, 2001.

8. Polynucleotides Encoding Insulin Homolog Zins3. Darrell Conklin, **Cathy Lofton-Day**, Si Lok, Steve Jaspers. ZymoGenetics, Inc. USA Patent #6,046,028, issued April 4, 2000.

**Patent Applications (published)**

Insulin Family Homolog Localized to Chromosome 1. Steve Jaspers, Ted Whitmore, Darrell Conklin, **Catherine Lofton-Day**, Si Lok. USSN 09/250,125.

Human Polypeptide Having Multiple EGF-Like Domains. Jim Holloway, **Catherine Lofton-Day**, and Teresa Gilbert. USSN 60/117,204.

Secreted Alpha-helical Protein ZLMDA24. Darrell Conklin, Zeren Gao, **Catherine E. Lofton-Day**, Theodore E. Whitmore. USSN 10/038,241.

UMLR Polypeptides. **Catherine E. Lofton-Day**, USSN 09/695, 369.

**Full-length Publications**

1. Strelkauskas, Anthony J. and **Catherine L. Taylor** (1986) Human monoclonal antibody: construction of stable clones reactive with human breast cancer. *Cancer Immunol. Immunother.* **23**:31-40.

2. Strelkauskas, Anthony J., **Catherine L. Taylor**, Matthew R. Smith, and Phyllis D. Bear (1987) Transfection of human cells: an alternative method for the establishment of human hybrid clones. In Human Hybridomas: Diagnostic and Therapeutic Applications. Marcel Dekker, Inc. A.J. Strelkauskas, ed.

3. Strelkauskas, Anthony J., **Catherine L. Taylor**, Paul H. Aldenderfer, and Glenn A. Warner (1987) Construction of stable human hybrid clones producing antibody reactive with human mammary carcinoma. In Human Hybridomas: Diagnostic and Therapeutic Applications. Marcel Dekker, Inc. A.J. Strelkauskas, ed.

4. Strelkauskas, Anthony J., and **Catherine L. Taylor** (1988) Human hybridomas: the road to the future. In The Lymphocyte-Structure and Function. Marcel Dekker, Inc. J.J. Marchalonis, ed.

5. Strelkauskas, Anthony J., **Catherine Lofton**, and Paul H. Aldenderfer (1987) Human monoclonal antibody: 2. Simultaneous expression of IgG and IgM with similar binding specificities by a human hybrid clone. *Hybridoma.* **6**: 479-487.

6. **Lofton, Catherine E.**, David A. Baron, William F. Oehlenschlager, and Mark G. Currie (1988) Pulmonary atrial natriuretic peptide. In Biological and Molecular Aspects of Atrial Factors. UCLA Symp. on Molecular and Cellular Biology. Volume 81, Phillip Needleman, ed.

7. Currie, M.G., **Catherine E. Lofton**, Howard Schomer, Walter H. Newman, Robert Wrenn, and William F. Oehlenschlager (1988) Pharmacology of the cardiac endocrine system. In Functional Morphology of the Endocrine Heart. W.G. Forssmann, ed.

8. Baron, D.A., **Catherine E. Lofton**, Walter H. Newman, and Mark G. Currie (1989) Atriopeptin inhibition of thrombin mediated changes in the morphology and permeability of endothelial monolayers. *Proc. Nat. Acad. Sci. USA* **86**: 3394-3398.

9. **Lofton, Catherine E.**, Walter H. Newman, and Mark G. Currie (1990) Atrial natriuretic peptide regulation of monolayer permeability is mediated by cGMP. *Biochem. Biophys. Res. Commun.* 172: 793-799.

10. **Lofton, Catherine E.**, David A. Baron, Walter H. Newman, John E. Heffner, and Mark G. Currie (1991). Atrial natriuretic peptide inhibits oxidant-induced increases in endothelial permeability and pulmonary edema. *J. Mol. Cell. Cardiology,* 23: 919-927.

11. Day, Joseph R., Patrick J. O'Hara, Francis J. Grant, **Catherine Lofton-Day**, Mary N. Berkaw, Phillip Werner, and Philippe Arnaud (1992). Molecular cloning and sequence analysis of the cDNA encoding human Apolipoprotein H (?-2-Glycoprotein I). *Int. J. Clin. Lab Res.* 21: 256-263.

12. Day, Joseph R., John J. Albers, **Catherine E. Lofton-Day**, Teresa Gilbert, Andrew F. Ching, Francis J. Grant, Patrick J. O'Hara, Santica M. Marcovina, and Janet L. Adolphson (1994). Complete cDNA encoding human phospholipid transfer protein from human endothelial cells. *J. Biol. Chem.* 269 (12): 9388-9391.

13. Lok, Si, Kenneth Kaushansky, Richard D. Holly, Joseph L. Kuijper, **Catherine E. Lofton-Day** et al. (1994). Cloning and expression of murine thrombopoietin cDNA and stimulation of platelet production *in vivo*. Nature 369: 565-568.

14. Kaushansky, Kenneth, Virginia C. Broudy, Nancy Lin, John McCarty, Maria Jorgensen, Norma Fox, Colleen O'Rork, and **Catherine Lofton-Day** (1995) Thrombopoietin, the mpl-ligand, is essential for full megakaryocyte development. Proc. Natl. Acad. Sci. 92: 3234-3238.

15. Weigle, David S., Thomas R. Bukowski, Donald C. Foster, Susan Holderman, Janet M. Kramer, Gerry Lasser, **Catherine E. Lofton-Day**, Donna E. Prunkard, Christopher Raymond and Joseph L. Kuijper. (1995) Recombinant *ob* protein reduces feeding and body weight in the *ob/ob* mouse. J. Clin. Inves. 96 (4): 2065-2070.

16. Kaushansky, K., V.C. Broudy, E. Sitnicka, **C. Lofton-Day**, A. Grossman, and K. Sprugel. (1996) Do the preclinical effects of thrombopoietin correlate with its in vitro properties? Stem Cells 14: Supplement 1.

17. LaGasse, James, Laura Jelinek, Shannon Sexson, **Cathy Lofton-Day**, John Breininger, Paul O. Sheppard, Wayne Kinsdvogel, and William A. Hagopian. (1997) An islet-cell tyrosine phosphatase is a likely precursor to the 37kDa autoantigen in Type 1 Diabetes: Human and macaque sequences, tissue distribution, unique and shared epitopes, and predictive autoantibodies. Molecular Medicine, 3 (3): 163-173.

18. Millar, R, D. Conklin, **C. Lofton-Day**, E. Hutchinson, B. Troskie, N. Illing. S.C. Sealfon and J. Hapgood (1999). A novel human GnRH receptor homolog gene: abundant and wide tissue distribution of the antisense transcript. Journal of Endocrinology, 162, 117-126.

19. Conklin, Darrell, **Catherine E. Lofton-Day**, Betty A. Haldeman, Andrew Ching, Theodore E. Whitmore, Si Lok and Stephen Jaspers (1999). Identification of INSL5, a new member of the insulin superfamily, Genomics 60 (1): 50-56.

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21. Gross, Jane, Janet Johnston, Sherri Mudri, Rachel Enselman, Stacey R. Dillon, Karen Madden, Wenfeng Xu, Julia Parrish-Novak, Don Foster, **Cathy Lofton-Day**, Margaret Moore, Alisa Littau, Angelika Grossman, Harald Haugen, Kevin Foley, Hal Blumberg, Kim Harrison, Wayne Kindsvogel, and Christopher H. Clegg (2000). TACI and BCMA are receptors for a TNF homolog implicated in B cell autoimmune disease, Nature 404 (6781): 995-999.

21. Whitmore, Theodore E., Jim Holloway, **Catherine E. Lofton-Day**, Mark F. Maurer, Lennie Chen, John Vincent, Stephen W. Scherer, and Si Lok (2000). Human secretin (SCT): Gene structure, chromosomal localization and distribution of mRNA, Cytogenetics and Cell Genetics 90: 47-52.

22. Parrish-Novak, Julia, Stacey R. Dillon, Andrew Nelson, Angie Hammond, Cindy Sprecher, Jane Gross, Janet Johnson, Karen Madden, Wenfeng Xu, Jim West, Sara Schrader, Steve Burkhead, Mark Heipel, Cameron Brandt, Joseph L. Kuijper, Janet Kramer, Darrell Conklin, Scott Presnell, Jon Berry, Faith Shiota, Susan Bort, Kevin Hambly, Sherri Mudri, Chris Clegg, Margaret Moore, Francis J. Grant, **Catherine Lofton-Day**, Teresa Gilbert, Fenella Raymond, Andrew Ching, Lena Yao, Deb Smith, Philippa Webster, Theodore Whitmore, Mark Maurer, Ken Kaushansky, Rick D. Holly, and Don Foster (2000). Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function Nature 408: 57-63.

23. Shi, Huidong, Pearlly S. Yan, Chuan-Mu Chen, Farahnaz Tahmatpanah, **Catherine Lofton-Day**, Charles W. Caldwell, and Tim Hui-Ming Huang (2002). Expressed CpG island sequence tag microarray for dual screening of DNA hypermethylation and gene silencing in cancer cells, Cancer Research 62: 3214-3220.